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Effect of supercoiling on the melting characteristics of heteropolynucleotides

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Supercoiled DNA melting; Cooperativity; Base-pair heterogeneity; Differential melting characteristics

Considering a supercoiled DNA molecule, having equal numbers of two distinct types of base-pairs, it has been shown theoretically that even for the extreme cases of mixing of the two types of base-pairs in a supercoiled DNA, the melting temperatures as well as the melting curves do not differ significantly. This indicates that these properties are practically independent of the detailed base sequence when the molecule is a covalently closed one and may be replaced by an equivalent homopolynucleotide whose binding energy is equal to the average base-pairing energy of the original DNA. This conclusion has been further supported by comparing the theoretical results with those obtained experimentally in the cases of polyoma DNA and ϕX174 DNA. Finally, the effects of supercoiling on the cooperativity of melting and a few aspects of the differential melting characteristics of a supercoiled DNA have been discussed which provide a clear physical understanding of the process.

1. Introduction

It is now well established that the melting transition of a supercoiled DNA differs significantly from that of its linear form. For example, a supercoiled DNA melts at a much higher temperature and over a wider temperature range than its linear counterpart. Recently, we have developed a statistical-mechanical theory for the melting transition in supercoiled homopolynucleotides [1]. The theory yields results in good agreement with the experimental data available for ϕX174 DNA melting in TEA (tetraethylammonium bromide) solution where a DNA behaves like a homopolymer. However, naturally occurring DNA molecules are not homopolymers and contain definite sequences of A-T and G-C base-pairs. The purpose of the present paper is to study theoretically the effects of base sequences on the melting characteristics. Experiments show that although DNA in its linear form exhibits strong sequence dependence of its melting property, the melting curve of the same DNA in its closed form is practically insensitive to its base sequence [2,3]. Our intention is to elucidate the underlying physical basis of this fundamental difference.

In the present treatement we have used practically the same approach based on the Ising model of a one-dimensional system that we used in the case of homopolynucleotides [1]. To discuss the effect of base sequence on the melting profile, we have considered a closed polynucleotide which contains equal numbers of two different types of base-pairs. These base-pairs can be distributed in a closed chain in a large number of ways. We have considered only the following two extreme cases of mixing: (i) every alternate position is occupied by the same type of base-pair so that mixing between the two types of base-pair is maximized; (ii) the successive base-pairs of one half of the

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molecule are of one type and the others are of the other type so that mixing is minimized. In each case, the melting profile has been computed by minimizing the total free energy of the molecule and the melting profiles for the two cases have been compared with each other. From the observation that the melting profiles even for these extreme cases of mixing differ only insignificantly from each other, we conclude that the melting transition of any real DNA molecule may be replaced by that of an equivalent homopolymer having the binding energy $\bar{\epsilon}$ which is equal to the average binding energy of the base-pairs of the original DNA. Applying this idea to the cases of melting of polyoma DNA and ϕX174 DNA in strongly chaotropic $NaClO_4$ solution, satisfactory agreement with the existing experimental data has been obtained. The differential melting characteristics (DMC), i.e., $d\theta/dT$ vs T plots, of the model DNA have also been studied in this paper. In the experimental studies of linear DNA, occasional sharp peaks are found in their DMC which are characteristic of the base sequences of the molecule and are generally known as the fine structure of the DMC. It is also an experimental fact that for the same DNA in ccc form, the fine structure is lost [2,3]. In the present paper, a clear physical reason behind this appearance of the fine structure in its DMC has been presented. It should be mentioned here that some theoretical investigations of this problem were undertaken earlier [4]. However, that approach suffers from the drawback that one cannot successfully incorporate into this theory the supercoiling energy which is present not only when the molecule has a non-zero initial supercoil density but also when a relaxed DNA melts. This has prompted us to investigate the problem from the standpoint of a more rigorous theory which properly accounts for the supercoiling energy.

2. Theory

2.1. Supercoiling energy

The linking difference ΔLk of a supercoiled DNA is distributed into twisting (Tw) and writhing (Wr) deformations of the double helix according to the relation $\Delta\mathrm{Lk} = \Delta\mathrm{Tw} + \mathrm{Wr}$, where writhing gives a measure of the bending of the central axis of the double helix [5,6]. On the basis of the elastic model of a supercoiled DNA [7–10], the twisting potential of each base-pair can be written as

$$v_i = \tfrac{1}{2} c_i \tau_i^2 \tag{1}$$

where c_i represents the twisting force constant of the i-th base pair of the chain and τ_i represents the twisting deformation of the i-th base-pair over its relaxed state value due to supercoiling. Obviously

$$\sum_i^N \tau_i = 2\pi\,\Delta\mathrm{Tw} \tag{2}$$

where ΔTw is the total twisting deformation and N represents the degree of polymerization of the DNA. The total twisting energy is then given by

$$v_\mathrm{T} = \sum_i v_i = \sum_i \tfrac{1}{2} c_i \tau_i^2 \tag{3}$$

Let us assume that c_i is independent of base-pair type [11] but is different for different secondary structures of the local region, i.e., $c_i = c_\mathrm{h}$ for a base-pair in the helix region of the DNA and $c_i = c_\mathrm{c}$ for a base-pair in the coiled (melted) region. Let τ_h and τ_c represent the rates of twisting deformations in the respective regions. Then for a DNA with n melted base-pairs we have

$$(N-n)\tau_\mathrm{h} + n\tau_\mathrm{c} = 2\pi\,\Delta\mathrm{Tw} \tag{4}$$

$$v_\mathrm{T} = \tfrac{1}{2}(N-n)c_\mathrm{h}\tau_\mathrm{h}^2 + \tfrac{1}{2}nc_\mathrm{c}\tau_\mathrm{c}^2 \tag{5}$$

Since a rigorous estimation of the writhing part is quite difficult, we have approximated it by assuming an interwound structure of supercoiled DNA to obtain a usable form in a manner similar to that of twisting. As a working hypothesis, the radius of curvature at each point of the bent molecule has been approximated by the radius of a circle whose circumference is equal to the contour length of each writhing turn [12,13]. Thus,

$$(N-n)\rho_\mathrm{h} + n\rho_\mathrm{c} = 2\pi\mathrm{Wr} \tag{6}$$

where ρ_h and ρ_c represent the average curvature rates per base-pair in the helix and coiled parts of

the DNA, respectively. Wr represents the number of writhing turns in the DNA molecule. The writhing energy arising from bending deformation is assumed to be expressed as

$$v_B = \sum \tfrac{1}{2} b_i \rho_i^2 \tag{7}$$

where b_i is the bending force constant of the i-th base-pair and ρ_i is the curvature at that base-pair. If b_h and b_c represent the bending constants of the helical and coiled regions, respectively, the writhing energy can be simplified as

$$v_B = \tfrac{1}{2}(N-n) b_h \rho_h^2 + \tfrac{1}{2} n b_c \rho_c^2 \tag{8}$$

The conservation of linking number Lk in a ccc DNA demands

$$\frac{N}{A} + \Delta\mathrm{Lk} = \frac{1}{2\pi}\left[(N-n)(\tau_h + \rho_h) + n(\tau_c + \rho_c)\right] + \frac{N-n}{A} \tag{9}$$

which, after simplification, gives

$$(N-n)(\tau_h + \rho_h) + n(\tau_c + \rho_c) = 2\pi\left(\Delta\mathrm{Lk} + \frac{n}{A}\right) \tag{10}$$

where A represents the number of base-pairs per helical turn of the B-form of DNA and is equal to 10.4 and $(N-n)/A$ thus represents the intrinsic linking number of the helical part of the DNA. ΔLk is the initial linking difference. From eqs 5 and 8, the total elastic energy of supercoiling is simply obtained as

$$G_s = v_B + v_T \tag{11}$$

Or,

$$G_s(\tau_h, \tau_c, \rho_h, \rho_c) = \tfrac{1}{2}(N-n)\left[c_h \tau_h^2 + b_h \rho_h^2\right] + \tfrac{1}{2} n\left[c_c \tau_c^2 + b_c \rho_c^2\right] \tag{12}$$

Let us minimize it with respect to the variables τ_h, τ_c, ρ_h and ρ_c for given values of N, n and ΔLk, under the constraint of linking number conservation given by eq. 10. Using Lagrange's method of undetermined multiplier for the extremization of $G_s(\tau_h, \tau_c, \rho_h, \rho_c)$, we obtain the following conditions

$$\frac{\partial G_s(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \tau_h} + \lambda \frac{\partial f(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \tau_h} = 0 \tag{13a}$$

$$\frac{\partial G_s(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \tau_c} + \lambda \frac{\partial f(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \tau_c} = 0 \tag{13b}$$

$$\frac{\partial G_s(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \rho_h} + \lambda \frac{\partial f(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \rho_h} = 0 \tag{13c}$$

$$\frac{\partial G_s(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \rho_c} + \lambda \frac{\partial f(\tau_h, \tau_c, \rho_h, \rho_c)}{\partial \rho_c} = 0 \tag{13d}$$

where

$$f(\tau_h, \tau_c, \rho_h, \rho_c) = 2\pi\left(\Delta\mathrm{Lk} + \frac{n}{A}\right) - (N-n)(\tau_h + \rho_h) - n(\tau_c + \rho_c) = 0 \tag{13e}$$

represents the contraint obtained from eq. 10, and λ is the associated multiplier. Eqs 13a–13d finally give

$$c_h \tau_h = c_c \tau_c = b_h \rho_h = b_c \rho_c = \lambda \tag{14}$$

Now, using eq. 14 we eliminate τ_c, ρ_c, and ρ_h from eq. 12 and obtain

$$G_s = \tfrac{1}{2}(b_h + c_h)\frac{c_h}{b_h}\left[N + (\alpha - 1)n\right]\tau_h^2 \tag{15}$$

$$\alpha = \frac{\alpha_c b_h + \alpha_b c_h}{b_h + c_h} \tag{16}$$

$\alpha_c = c_h/c_c$ and $\alpha_b = b_h/b_c$. Again, from eqs 10 and 14, it follows that

$$\tau_h = \frac{2\pi b_h\left[\Delta\mathrm{Lk} + \frac{n}{A}\right]}{(N-n)(b_h + c_h) + n\left[\alpha_c b_h + \alpha_b c_h\right]} \tag{17}$$

Substituting this value of τ_h in eq. 15 we finally obtain

$$G_s = C\frac{\left[\Delta\mathrm{Lk} + \frac{n}{A}\right]^2}{\left[N + (\alpha - 1)n\right]} \tag{18}$$

$$C = \frac{2\pi^2 b_h c_h}{(b_h + c_h)} \tag{19}$$

Thus, G_s is effectively a function of the two parameters C and α only. One should note that the functional form of the supercoiling energy expression does not depend on the exact partitioning of the effective linking difference into twisting ΔTw and writhing Wr because twisting and writh-

ing are related to the total linking number Lk through a linear relationship. It will only change the values of C and α which in no way affects the qualitative features.

Let us assume that the model closed circular DNA is N (an even number) base-pairs long of which $N/2$ is of type 1 and the rest is of type 2 base-pairs. We assume that the base-pair type is represented by the binding energy ϵ_i of the i-th base-pair. ϵ_i can take either of the two values, E_1 and E_2. Then for the following two distributions

$$(i)\ \epsilon_i = E_1 \text{ for } i = 1, 3, 5, \ldots 2N-1.$$
$$= E_2 \text{ for } i = 2, 4, 6, \ldots 2N.$$
$$(ii)\ \epsilon_i = E_1 \text{ for } i = 1, 2, 3, \ldots \frac{N}{2}.$$
$$= E_2 \text{ for } i = \frac{N}{2}+1, \ldots N$$

we calculate the melting characteristics as follows.

2.2. Free energy of melting

2.2.1. Case (i)

In this case the molecule is effectively a homopolynucleotide of average binding energy $\bar{\epsilon}$ given by

$$\bar{\epsilon} = \tfrac{1}{2}(E_1 + E_2) \tag{20}$$

When the molecule is partially melted, suppose there are n melted base-pairs in n_r melted regions creating n_j helix-coil junctions. Clearly, in a ccc DNA, n_j junctions divide the molecule into $(1/2)n_j$ $(=n_r)$ helical and an equal number of melted regions. It may be noted that for a covalently closed circular DNA, n_j is always even. The total free energy of the partially melted molecule is then simply given by

$$F = G_s(n, \Delta\mathrm{Lk}) + n(\bar{\epsilon} - T\Delta S) + n_r\epsilon_0 - kT \ln g(N,n,n_r) \tag{21}$$

where $G_s(n,\Delta\mathrm{Lk})$ represents the supercoiling energy part and is given by eq. 18. $(\bar{\epsilon} - T\Delta S)$ represents the free energy of a broken base-pair where ΔS is the change in entropy per base-pair due to melting so that the second term represents the total melting energy of the n melted base-pairs. In the next term, ϵ_0 is the nucleation energy of melting and is equal to the base-pair stacking energy. This energy arises due to the fact that, to initiate melting in an intact helical portion of the DNA molecule, an extra stacking energy is required to be broken. Finally, the last term represents the configurational free energy which appears due to the possibility of distributing the n melted base-pairs in n_r melted regions and $(N-n)$ bonded base-pairs in n_r helix regions in a large number of ways $g(N,n,n_r)$ with at least one base-pair per region. In our earlier paper [1], this degeneracy factor has been determined and is given by

$$g(N,n,n_r) = \frac{N(N-n-1)!(n-1)!}{(N-n-n_r)!(n_r-1)!(n-n_r)!n_r!} \tag{22}$$

For simplicity we will neglect 1 in the above equation and use Stirling's approximation in appropriate places. The equilibrium characteristics of the transition may now be obtained by minimizing the total free energy F as given by eq. 21 with respect to n and n_r. This leads to the conditions

$$N\theta(1-\theta)(\xi-1) - [\xi(1-\theta)-\theta]n_r = 0 \tag{23}$$

and

$$N\left[1 - \{1 - 4\eta\theta(1-\theta)\}^{1/2}\right] - 2\eta n_r = 0 \tag{24}$$

where

$$\xi = \exp[(\bar{\epsilon} - T\Delta S + \gamma)/kT] \tag{25}$$

$$\eta = 1 - \exp(\epsilon_0/kT) \tag{26}$$

$$\gamma = \frac{\partial G_s}{\partial n} \tag{27}$$

and

$$\theta = \frac{n}{N} \tag{28}$$

Here, θ represents the melted fraction of base-pairs in the molecule. Eliminating n_r from eqs 23 and 24, we finally obtain

$$2\eta\theta(1-\theta)(\xi-1) - [\xi(1-\theta)-\theta] \times \left[1 - \{1 - 4\eta\theta(1-\theta)\}^{1/2}\right] = 0 \tag{29}$$

which can be solved iteratively to obtain the melting curve (θ vs T) in this case.

The melting temperature is obtained by solving eq. 29 with $\theta = 1/2$ which defines the melting point T_m. Thus,

$$T_m = T_m^1 + \Delta T_m \tag{30}$$

where $T_m^1 = \bar{\epsilon}/\Delta S$ represents the melting temperature of the DNA molecule in its linear form and

$$\Delta T_m(\sigma) = \frac{\gamma_m(\sigma)}{\Delta S} = \frac{C(2\sigma+1)[(\alpha-1)(1-2\sigma)+4]}{A^2(\alpha+1)^2\,\Delta S} \tag{31}$$

represents the change in melting temperature due to supercoiling. Here, $\sigma = A\,\Delta Lk/N$ is the initial supercoil density of the ccc DNA.

2.2.2. Case (ii)

In this case as already mentioned, the base-pairs of the hypothetical molecule are redistributed along the chain such that half of it contains successive base-pairs of type 1 only, while the remaining half contains the base-pairs of type 2. Let, at a particular temperature T, n_1 base-pairs in segment 1 (containing type 1 base-pairs only) melt and create n_{r1} melted regions there. Similarly, n_2 base-pairs are melted in segment 2 (containing type 2 base-pairs only) creating n_{r2} melted regions. Then the total free energy of the partially melted molecule is given by

$$F = (n_{r1}+n_{r2})\epsilon_0 + n_1(E_1 - T\Delta S) + n_2(E_2 - T\Delta S) + G_s(n_1,n_2,\sigma,N) - kT\,\ln\, g(n_1,n_2,n_{r1},n_{r2},N) \tag{32}$$

In the above equation, the first term represents the total nucleation energy of the molecule and the next two terms give the total increase in free energy due to melting of n_1 type 1 base-pairs in segment 1 and n_2 type 2 base-pairs in segment 2, respectively. G_s represents the overall supercoiling energy in the whole molecule and is clearly given by eq. 18 with $n = n_1 + n_2$. It should be noted that through G_s the melting processes of the two segments of the molecule become coupled together. The last term corresponds to the configurational free energy. Here, $g(n_1,n_2,n_{r1},n_{r2})$ represents the degeneracy factor. The exact analytical evaluation of the degeneracy factor in this case is quite difficult. Therefore, we have estimated it by the following approximation. Since in a large molecule the states of the two junctions between the two segments should not influence the bulk behaviour of melting, we can consider the distributions of the helical and the melted regions over the entire chain as the product of two independent distributions $g_1(n_1,n_{r1},N')$ and $g_2(n_2,n_{r2},N')$

$$g(n_1,n_2,n_{r1},n_{r2},N') = g_1(n_1,n_{r1},N')\cdot g_2(n_2,n_{r2},N') \tag{33}$$

where

$$g_1(n_1,n_{r1},N') = \frac{2(n_1-1)!(N'-n_1-1)!}{(n_1-n_{r1})!(N'-n_1-n_{r1})!(n_{r1}-1)!(n_{r1}-1)!} \tag{34a}$$

represents the number of ways of distributions of the n_1 melted base-pairs in the n_{r1} melted regions in segment 1 with at least one base-pair per region [1,14,15]. Here, $N' = N/2$. Similarly,

$$g_2(n_2,n_{r2},N') = \frac{2(n_2-1)!(N'-n_2-1)!}{(n_2-n_{r2})!(N'-n_{r2}-n_2)!(n_{r2}-1)!(n_{r2}-1)!} \tag{34b}$$

represents the number of ways of distribution of n_2 base-pairs in n_{r2} melted regions in segment 2 with at least one base-pair per region. Assuming $n_1,n_2,n_{r1},n_{r2} \gg 1$, we may neglect the 1s in eqs 34a and 34b. Now, if we minimize the total free energy F given by eq. 32 with respect to n_1,n_2,n_{r1},n_{r2}, we finally obtain

$$N\theta_i(1-\theta_i)(\xi_i-1) - 2[(1-\theta_i)\xi_i - \theta_i]\,n_{ri} = 0, \qquad i = 1,2 \tag{35}$$

$$N\left[1 - \{1 - 4\eta\theta_i(1-\theta_i)\}^{1/2}\right] - 4\eta n_{ri} = 0, \quad i = 1,2 \tag{36}$$

where

$$\xi_i = \exp[(E_i - T\Delta S + \gamma_i)/kT], \quad i = 1,2 \tag{37}$$

$$\gamma_i = \frac{\partial G_s}{\partial n_i} \tag{38}$$

and

$$\theta_i = 2n_i/N \tag{39}$$

and η is given by eq. 26. Eliminating n_{r1} and n_{r2} from the above equations, we finally obtain

$$2\eta\theta_1(1-\theta_1)(\xi_1-1) - [\xi_1(1-\theta_1)-\theta_1]\left[1-\{1-4\eta\theta_1(1-\theta_1)\}^{1/2}\right] = 0 \tag{40}$$

$$2\eta\theta_2(1-\theta_2)(\xi_2-1) - [\xi_2(1-\theta_2)-\theta_2]\left[1-\{1-4\eta\theta_2(1-\theta_2)\}^{1/2}\right] = 0 \tag{41}$$

It may be noted that these two equations are coupled through ξ_1 and ξ_2 which are functions of $(\theta_1+\theta_2)$. These two simultaneous equations can be solved numerically by the Newton-Raphson method for θ_1 and θ_2 and the overall melting curve (θ vs T) can be obtained where

$$\theta = \tfrac{1}{2}(\theta_1+\theta_2) \tag{42}$$

represents the overall melted fraction of base-pairs.

3. Results and discussions

In the numerical calculations we have used the standard values of the parameters as $\Delta S = 12$ e.u. [16] and $\epsilon_0 = 2.5$ kcal/mol [17,18] for all the cases under consideration. Since the values of the elastic parameters (b_h, c_h, b_c and c_c) are not accurately known, we have chosen the values of the effective parameters C and α as 9.4×10^{-11} erg and 23.4, respectively, to fit the melting curve of polyoma DNA in 7.2 M $NaClO_4$ solution. For the closed double-stranded hypothetical molecule under consideration, we have taken $E_1 = 7.7$ kcal/mol and $E_2 = 8.2$ kcal/mol as model parameters for the first set, giving a value for $(T_{m2} - T_{m1}) = 20\,^\circ$C comparable to the melting range of supercoiled DNA. With these values we have solved eqs 40 and 41 numerically by the Newton-Raphson method for θ_1 and θ_2 according to case (ii). The overall melting curve is then plotted as the solid line in fig. 1. Again considering the molecule as a homopolymer of average base-pairing energy $\bar{\epsilon}$ given by eq. 20 for the base sequence according to case (i), eq. 29 has been solved and the resulting

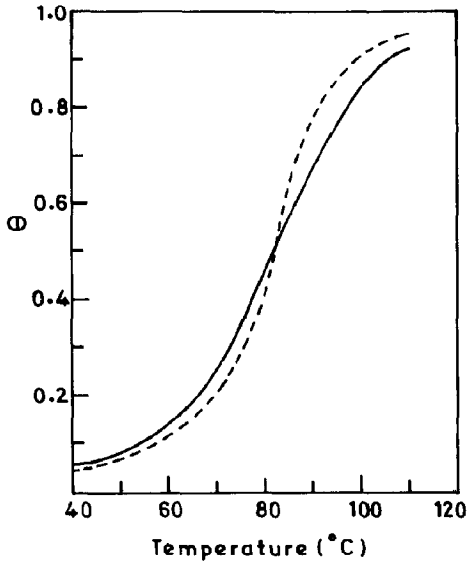

Fig. 1. Melting curves for the hypothetical supercoiled DNA (see text) when the molecule corresponds to case (i) (dashed line) and case (ii) (solid line). The binding energies of the two types of base-pairs are such that $T_{m2} - T_{m1} = 20\,^\circ$C, and $\sigma = -0.05$. Clearly, the melting curves in the two situations are nearly identical, indicating that the detailed base sequence has little effect on melting in the case of a supercoiled DNA.

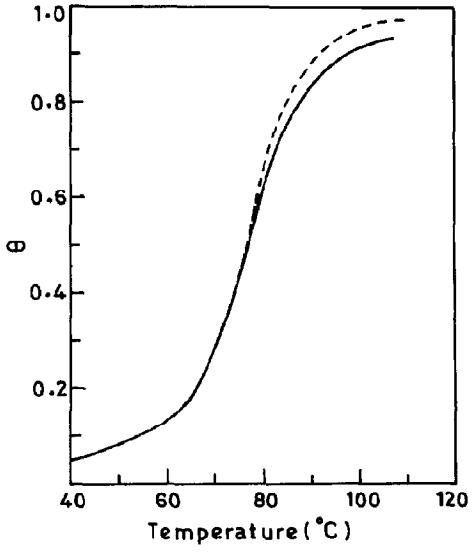

Fig. 2. Same as fig. 1 except that the binding energy difference between the two types of base-pairs has been decreased ($T_{m2} - T_{m1} = 10\,^\circ$C). Obviously the agreement between the two curves in this case is better.

melting curve is shown by the broken line in fig. 1. It is important to note that the melting temperature as well as the melting curve in these two extreme cases are nearly identical although the base sequence in case (ii) has the minimum likeness to a homopolymer. The other set of such plots (fig. 2) represent the case where $(T_{m2} - T_{m1}) = 10\,^{\circ}C$. Clearly, for lower values of $(T_{m2} - T_{m1})$, better agreement between the two curves is obtained. The above analysis thus gives a theoretical reproduction of the experimentally observed feature that in a ccc DNA melting base sequence is rather unimportant. We further conclude that in such cases the molecule may be considered as an equivalent homopolymer of base-pairing energy $\bar{\epsilon}$ which is the average base-pairing energy of the original molecule and clearly depends on its base content. This conclusion is also supported by our results for polyoma DNA (fig. 3) and for ϕX174 DNA (fig. 4) in $NaClO_4$ solution by treating these molecules as effective homopolymers. The average binding energy $\bar{\epsilon}$ is obtained from the relation $\bar{\epsilon} - T_m^1 \Delta S = 0$. Using $T_m^1 = 48\,^{\circ}C$ for polyoma DNA [19] and $T_m^1 = 47\,^{\circ}C$ for ϕX174 DNA [2] in $NaClO_4$ solution, we obtain $\bar{\epsilon} = 7.70$ and 7.68 kcal/mol, respectively. The supercoiling density σ is -0.063 for ϕX174 DNA and -0.032 for polyoma DNA. The agreement between the theoretical and experimental curves is found to be quite satisfactory.

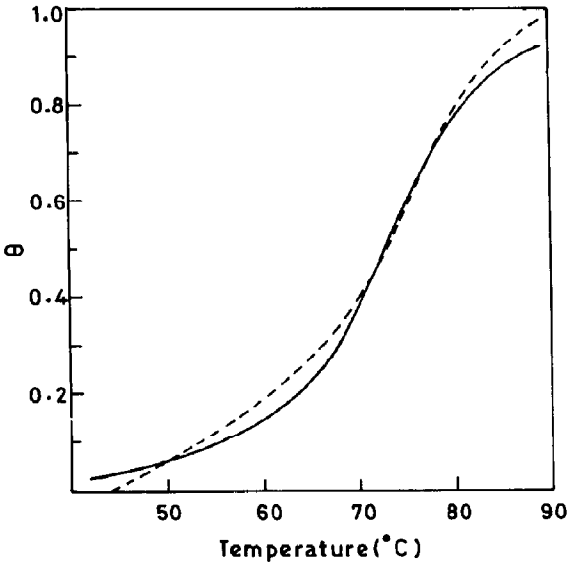

Fig. 3. Comparison of the theoretical melting curve (solid line) for supercoiled polyoma DNA ($\sigma = -0.032$), with the corresponding experimental data in 7.2 M $NaClO_4$ solution (dashed line).

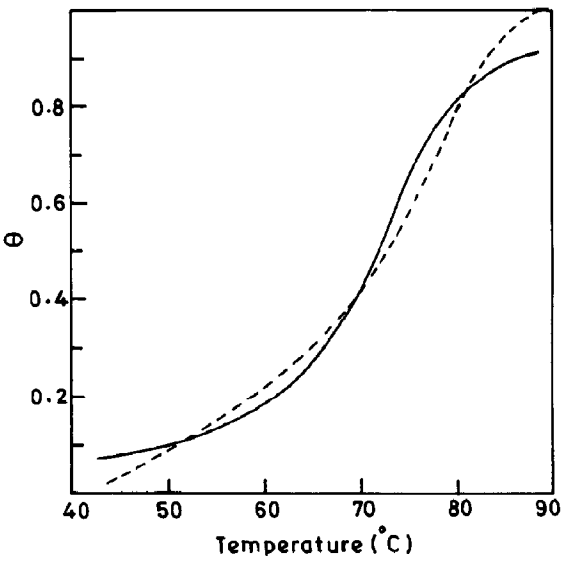

Fig. 4. Theoretical (solid line) and experimental (dashed line) melting curves for supercoiled ϕX174 DNA ($\sigma = -0.063$) in 7.2 M $NaClO_4$ solution.

It is also observed from the experimental data that the change in melting temperature ΔT_m due to supercoiling is hardly dependent on the base-pair heterogeneity of the molecule [2]. This behaviour is, however, expected in the light of eq. 31 which indicates that ΔT_m depends on C, α and σ and is independent of $\bar{\epsilon}$ which is the only parameter that depends on the base composition of a DNA. Thus, for a DNA molecule with a given value of σ, ΔT_m depends only on α and C which are determined by the secondary structure of the molecule (eqs 16 and 19) and likely to be independent of its primary structure (base sequence as well as base content). Therefore, it seems quite natural that ΔT_m also should not be dependent on base-pair heterogeneity. However, the dependence of the overall melting temperature T_m $(= T_m^1 + \Delta T_m)$ on heterogeneity comes through $T_m^1 = \bar{\epsilon}/\Delta S$ where $\bar{\epsilon}$ is strongly dependent on the base content.

To study the effect of supercoiling on the cooperativity of the transition, we have calculated

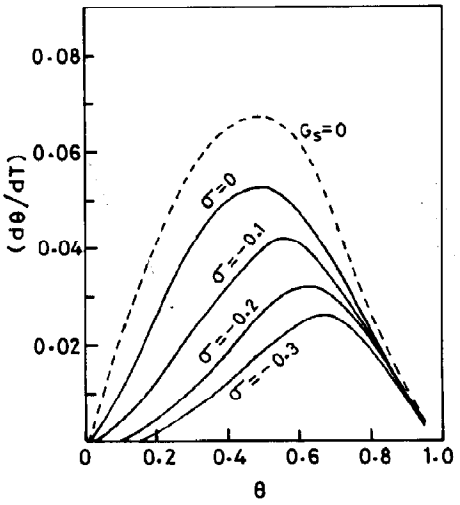

Fig. 5. Computed curves representing $d\theta/dT$ vs θ (solid lines) for various supercoiling densities σ. The dashed curve represents the case where the supercoiling energy is artificially made zero. These curves clearly show that the $d\theta/dT$ values decrease over the entire range of melting as supercoil density increases, which indicates that supercoiling has an anticooperative action on melting.

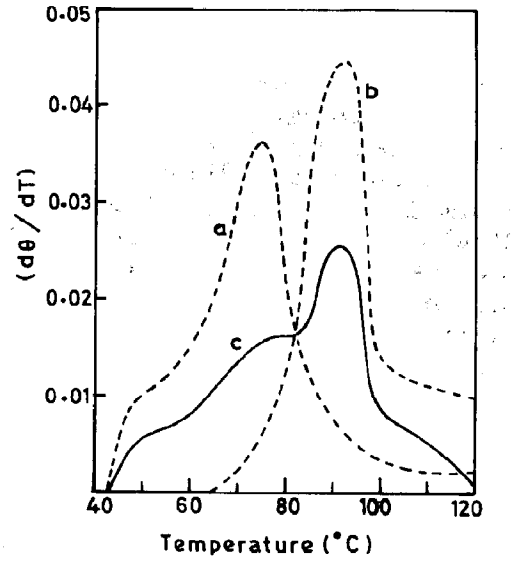

Fig. 6. Computed differential melting curve of the hypothetical DNA ($T_{m2} - T_{m1} = 20\,°C$) for case (ii) (solid line c). The dashed curves (a, b) represent the individual DMCs of segment 1 and 2. It is evident that DMCs (a, b) are sufficiently broad and overlap considerably.

numerically the values of $d\theta/dT$, the temperature rate of melting and plotted the ($d\theta/dT$ vs θ) curves for different values of initial supercoil density σ. Clearly, higher values of $d\theta/dT$ correspond to greater cooperativity. Comparison of such $d\theta/dT$ vs θ curves for different σ values gives information about the effects of σ on the cooperativity of the transition. Fig. 5 shows such curves. The dashed curve corresponds to the case where the effectively long-range (supercoiling) interaction part is artificially maintained at zero throughout the transition so that it cannot contribute to the process of melting. The first solid curve represents the case ($\sigma = 0$) where effective positive supercoiling is generated in the molecule due to melting. Comparison between these two curves shows that supercoiling has a strong anticooperative action on the melting process and as a result the melting curve becomes flatter. With increasing initial supercoil density, the anticooperative action increases as is indicated by the curves in fig. 5.

Finally, the differential melting curves for the hypothetical molecule in case (ii) have been com-

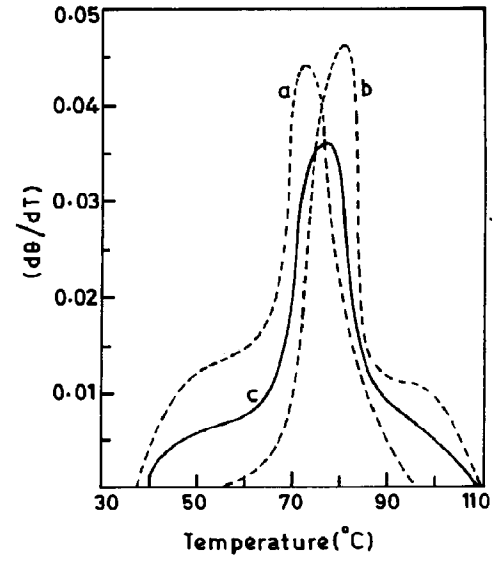

Fig. 7. Same as fig. 6 except that the binding energy difference between the two types of base-pairs is reduced ($T_{m2} - T_{m1} = 10\,°C$). Large overlapping (compared to fig. 6) of the DMCs of the segments is found.

puted numerically and are shown in figs 6 and 7. It is observed that the individual DMCs for θ_1 and θ_2 are very flat as is expected according to the above discussions and overlap considerably even for high values of $(T_{m2} - T_{m1})$. This observation provides a basis for the qualitative understanding of the nature of the DMC of a supercoiled DNA. It is known that depending on the local base content, a DNA molecule may be considered as a succession of several zones of distinct average binding energies [20,21]. Some of these zones may have the same average binding energy and thus form degenerate zones. Hence, for the different average binding energies, there may be sets of degenerate zones. Each set of such zones corresponding to each energy melts about the respective melting temperature giving a peak in its DMC. In the case of a linear DNA the high degree of cooperativity (melting is completed over a few degrees only) prevents these peaks from overlapping and thus appear distinctly in the corresponding DMC. However, in the case of a closed DNA, since the transition is found to occur over a wide temperature range (as demonstrated in the figures) the different peaks are smeared into broad humps and overlap considerably. As a result the fine structure is lost.

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